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New insights in Hg and Se speciation in the rainbow trout, an aquaculture model

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Metal/metalloid species and their molecular characterization

- ✓ Identify the metal species to evaluate their **role** in metal biogeochemical/biological cycling
- ✓ **Hg²⁺ and MeHg** fate/toxicity/reactivity rely on their **molecular targets**: presence of **metal complexes**
- ✓ Potential **Hg detoxification** by selenium (Se) depends also on **Se chemical form**

- **Little is known** => “infinite” possibilities
- **Unusual**, potentially **unstable** compounds with **hard to predict** reactivity
- Frequently at **trace levels** (ppb) (masked by unmetallated organic compounds)

⇒ **Need for the development of a systematic approach to determine amount and identity of metal species using analytical chemistry tools**

 **Speciation analysis => LC MS**

Understanding mercury-selenium interactions in fish

Evaluate mercury burden in rainbow trouts fed with tuna byproducts based diet

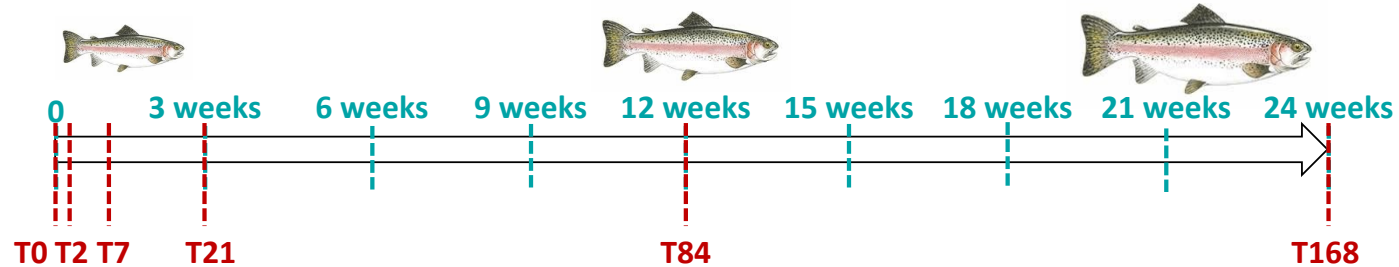
2nd most produced fish species in Europe

Tuna-based diet
or
Plant-based diet

± MeHg (~2 µg/g)
± Se(IV) (~1.5 µg/g)
± Se-Methionine (~1.5 µg/g)



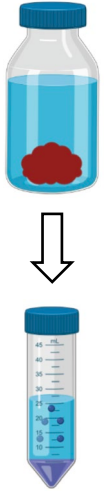
Brain
Liver
Muscle
Kidney
Blood



Focus on determining Hg molecular targets in parallel to Se uptake

Mass spectrometry methodology for Hg and Se molecular characterization

Sample preparation & separation methods

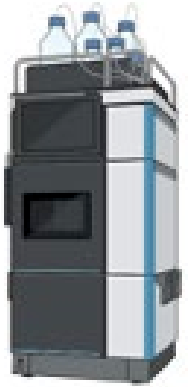


Optimized Hg and Se compounds extraction:

- Check compound stability during extraction and after extraction
- Check chromatographic recovery

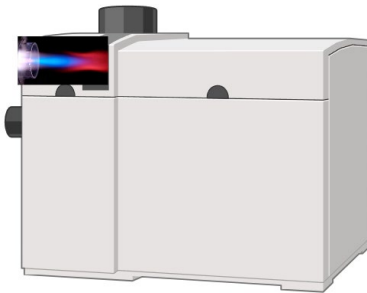
Compared liquid chromatographic methods:

- Hydrophilic interaction chromatography (HILIC)
- Size Exclusion Chromatography (SEC)



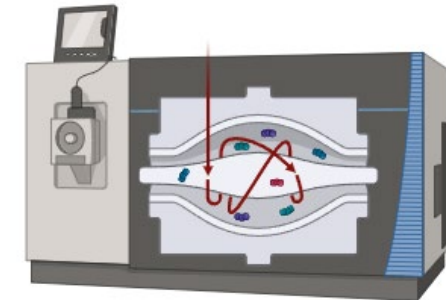
Detection methods

Elemental mass spectrometry (ICP-MS)



After the atomization of all the constituents of the sample, allow the determination of metal/metalloid containing compounds

Molecular mass spectrometry (ESI-FTMS)

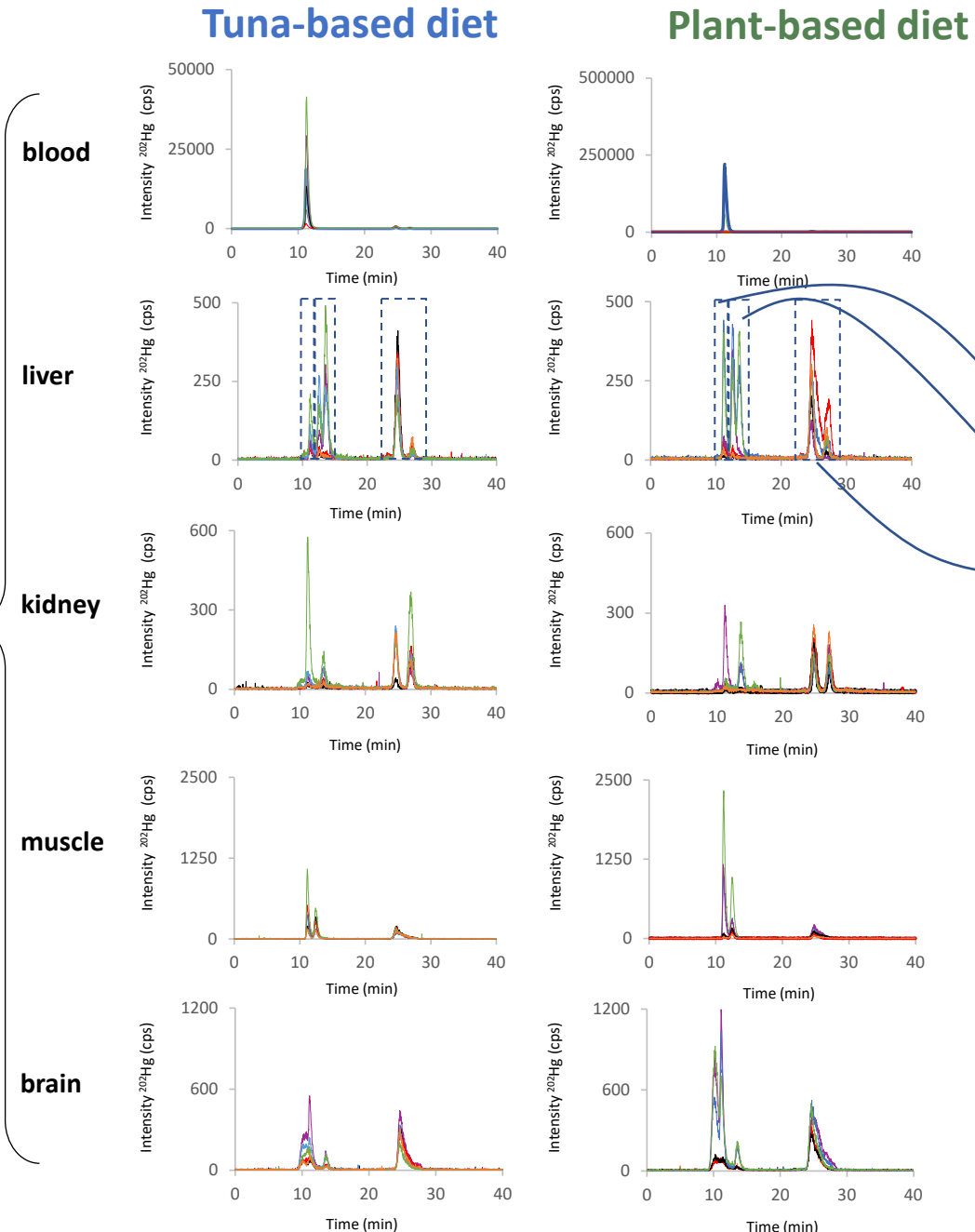


With soft ionization processes (ESI), allow the identification of the intact molecules in presence according to their m/z

Hg speciation in trout by LC MS techniques

- Analysis of water soluble fraction from rainbow trout exposed during 168 days

SECpeptide-ICP-MS chromatograms of ²⁰²Hg

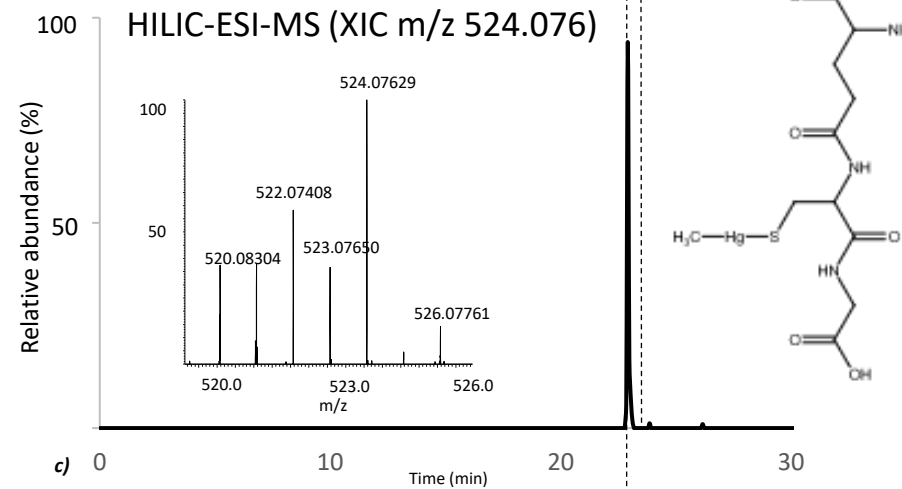
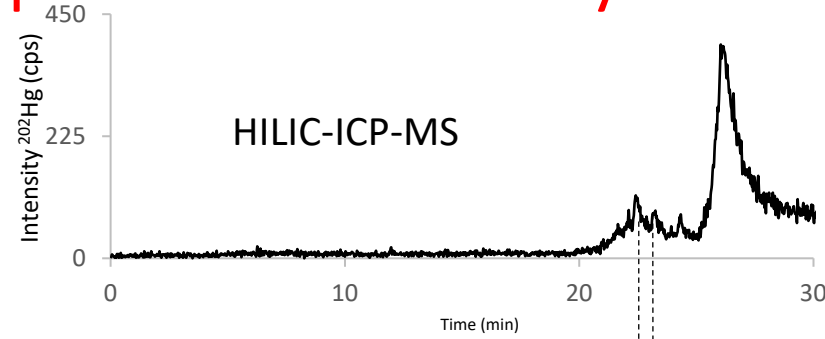


Hg speciation in trout by LC MS techniques

- 0.1-1 kDa fraction was also detected by HILIC MS couplings

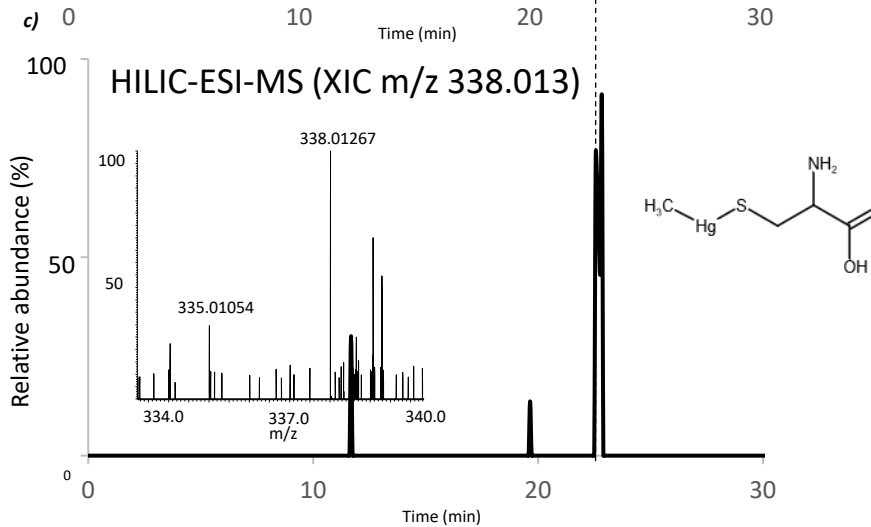
Glutathione (GSH)

GSH-MeHg complex



Cysteine (Cys)

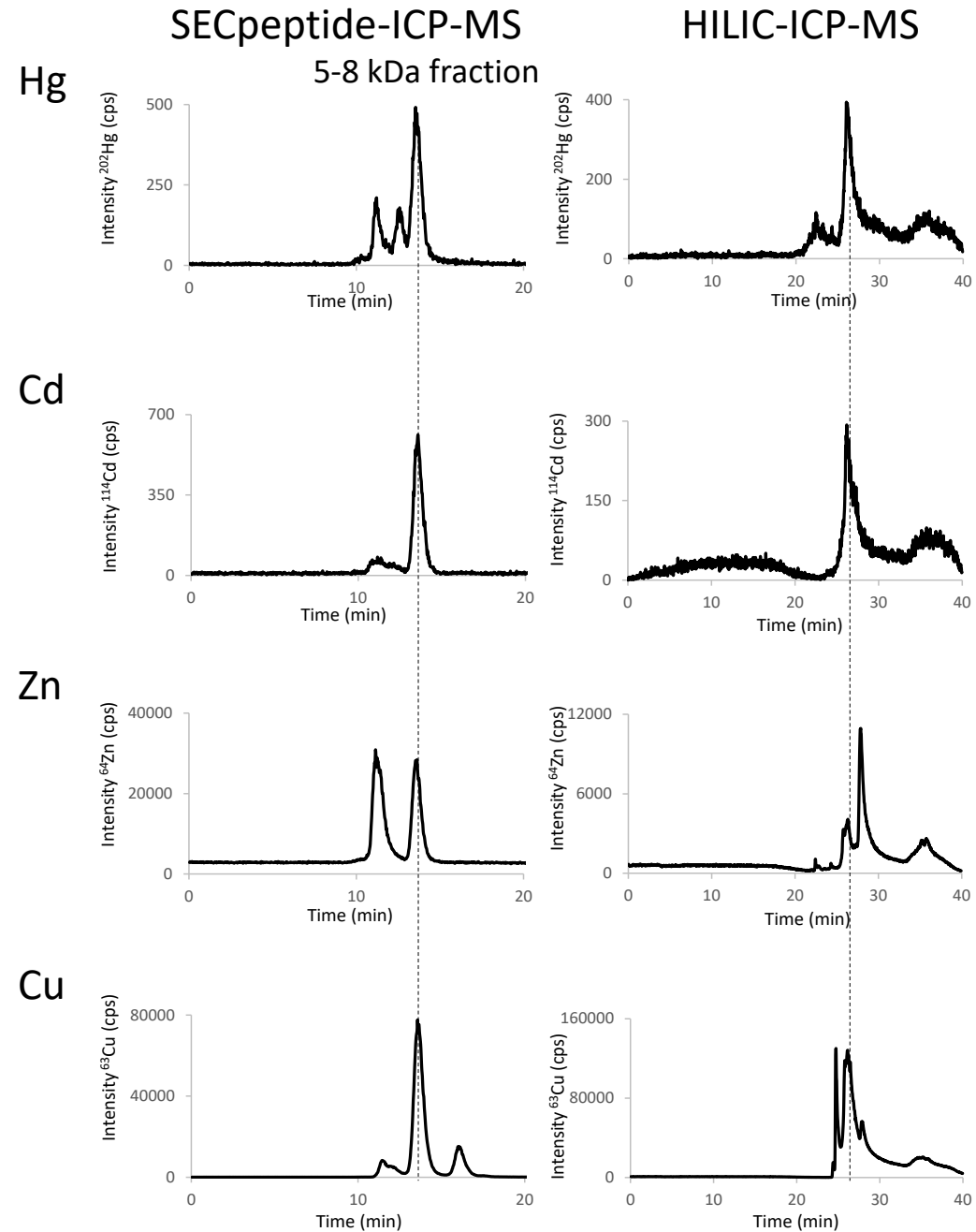
Cys-MeHg complex



✓ LMW thiols involved in MeHg binding

Hg speciation in trout by LC MS techniques

- Comparison with other elements

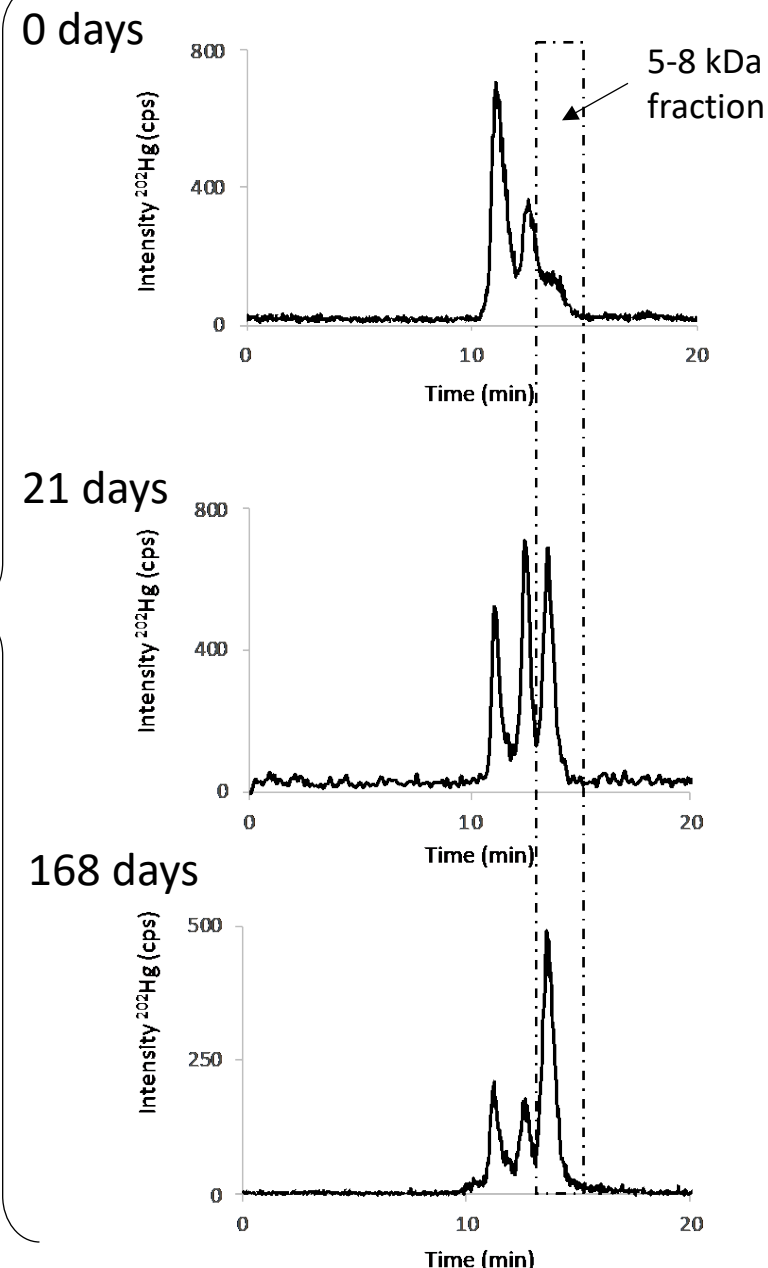


✓ 5-8 kDa molecule is involved in Hg, Cd, Zn and Cu binding

Hg speciation in trout by LC MS techniques

- Trend during exposition:
liver extract with tuna-based diet + MeHg

SECpeptide-ICP-MS chromatograms of ^{202}Hg

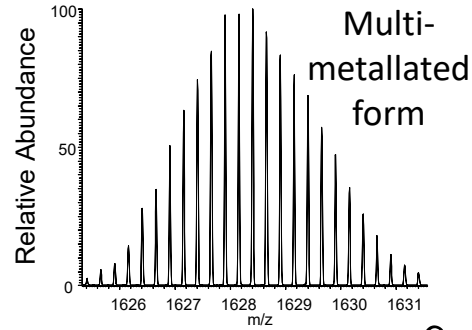
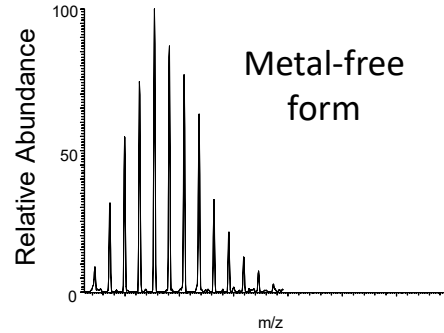


✓ The 5-8 kDa protein fraction becomes predominant over time

Hg speciation in trout by LC MS techniques

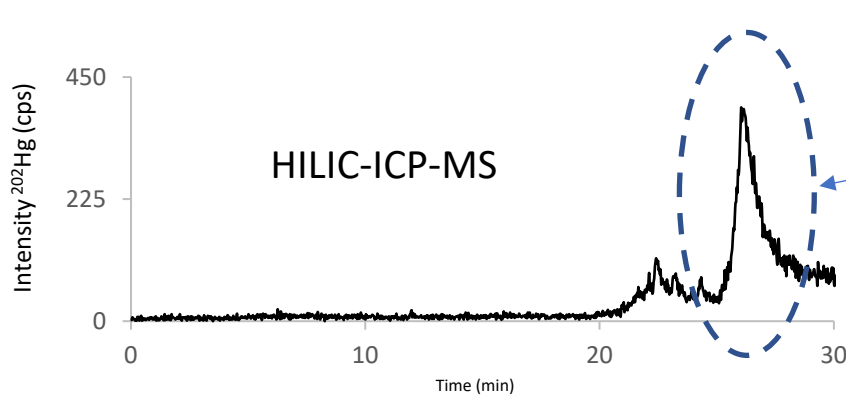
Cu, Zn, Cd and Hg are multi-isotopic elements

↳ A unusually broader isotopic pattern should be observed for these expected metal binding proteins



Detected trout proteins/peptides binding metal after HILIC-ESI-MS analysis

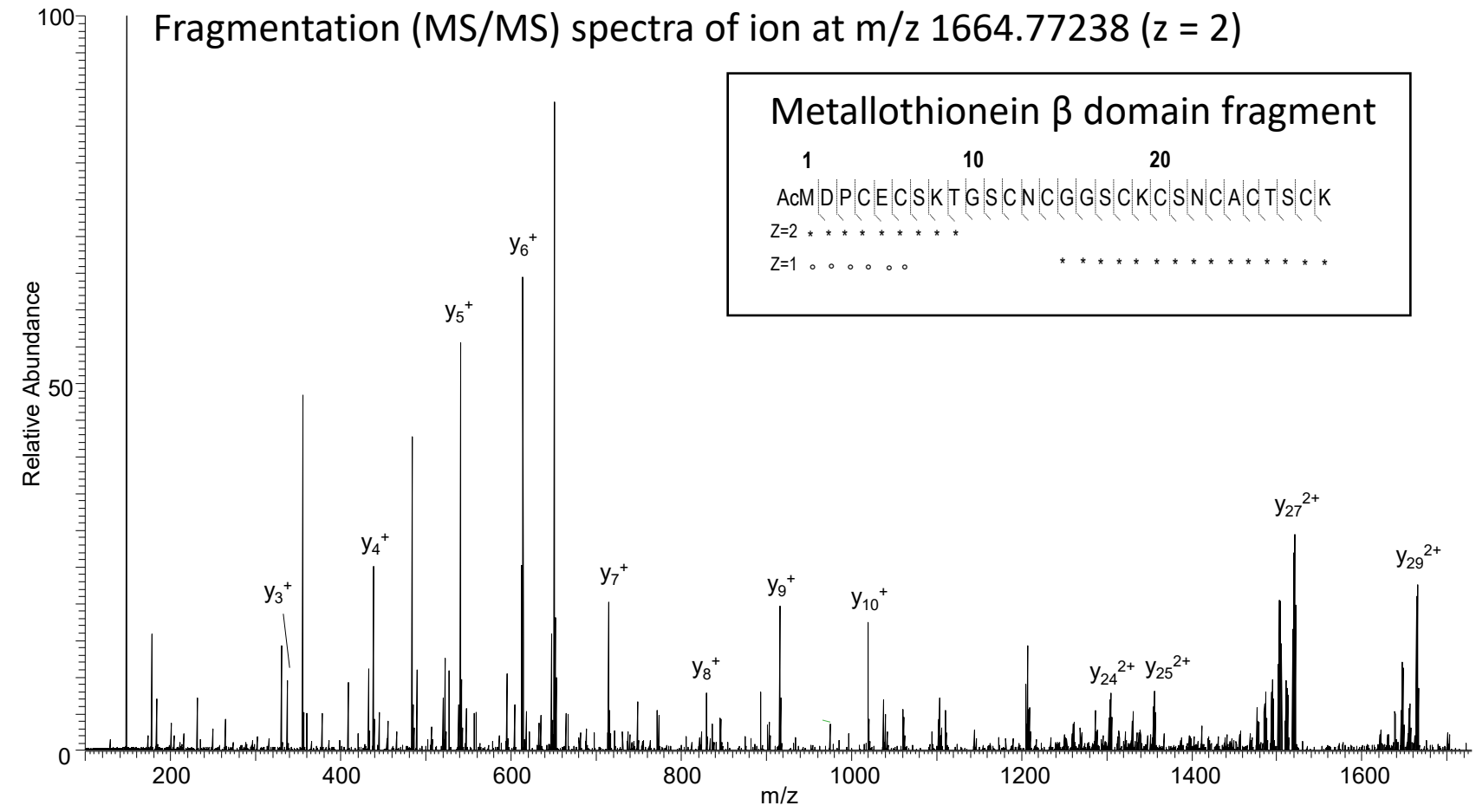
Retention time	m/z measured
26.5	1683.33490 (z=4)
26.0	1675.58372 (z=4)
24.9	1664.77238 (z=2)



Corresponding MS pattern

Corresponding retention time

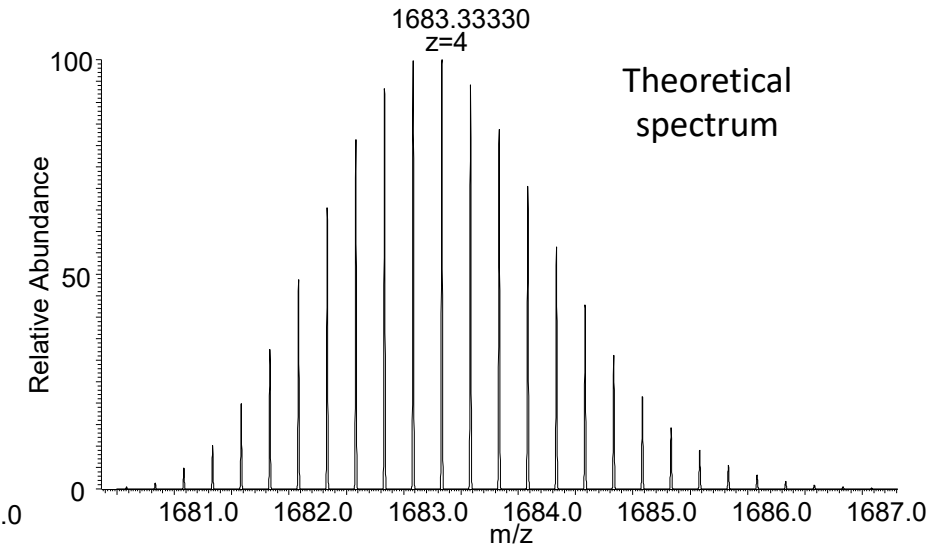
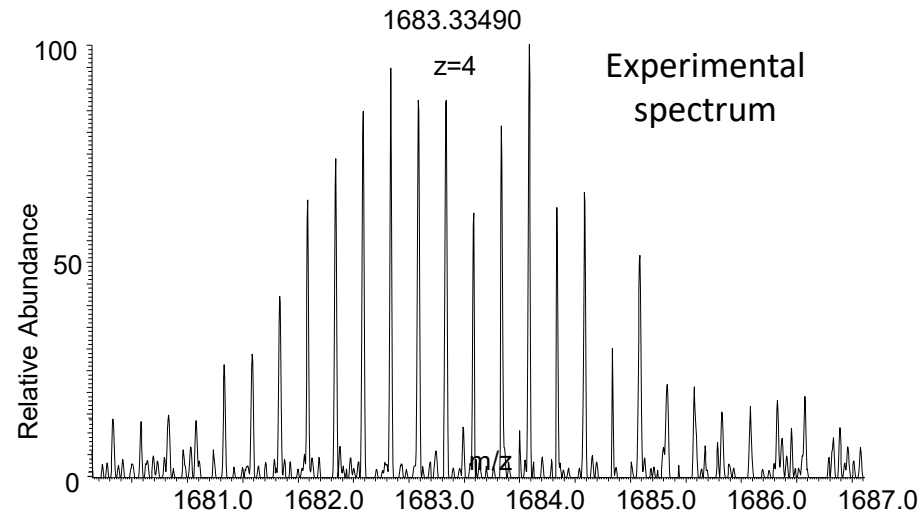
Hg speciation in trout by LC MS techniques



✓ Presence of metallothionein fragments

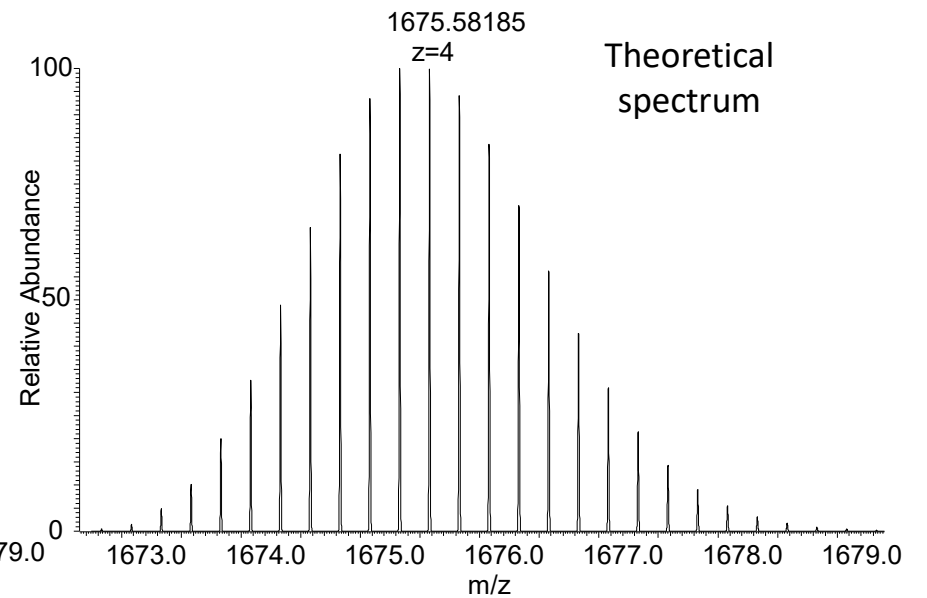
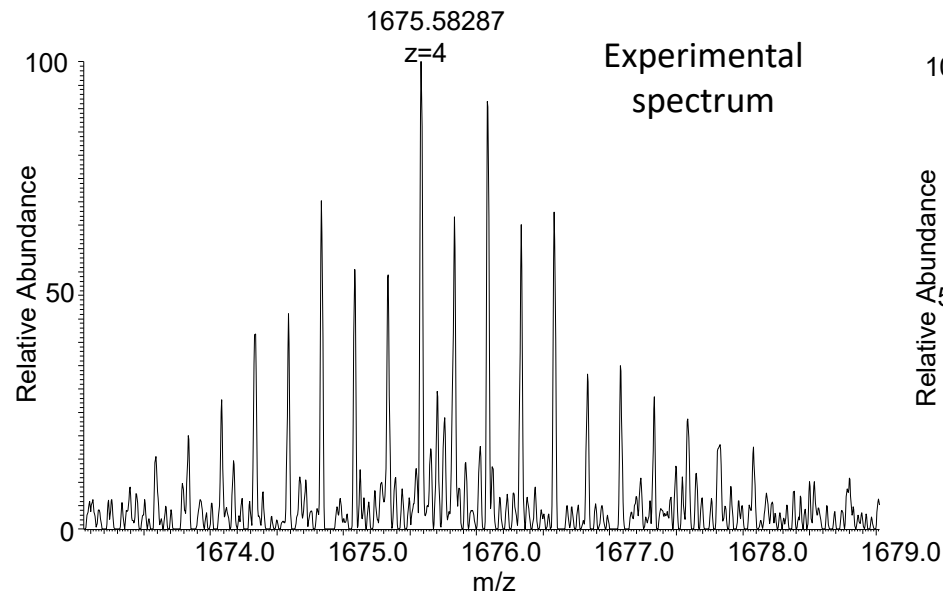
Hg speciation in trout by LC MS techniques

✓ Identification of trout metallothioneins A and B (MS profile similar to predicted ones and perfect mass matching)



Metallothionein A – 10 Cu(I) complex

AcMDPCECSKTGSCNCGGSKCSNCACTSCKKASCCDCCPSGCSKCASGCVCKGKTCDTSCCQ



Metallothionein B – 10 Cu(I) complex

AcMDPCECSKTGSCNCGGSKCSNCACTSCKKSCCPCPSDCSKCASGCVCKGKTCDTSCCQ

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Hg speciation in trout by LC MS techniques

Identification of trout MT isoforms or their fragments

Retention time	m/z measured	m/z theoretical	Mass difference (ppm)	Compound
26.5	1683.33490 (z=4)	1683.33330 (z=4)	+ 1.0	Cu ₁₀ -NAcMT _A
26.0	1675.58372 (z=4)	1675.58185 (z=4)	+ 1.1	Cu ₁₀ -NAcMT _B
24.9	1664.77238 (z=2)	1664.76977 (z=2)	+ 1.6	Cu ₆ -NAcMT _(A ou B) β domain

✓ Presence of trout metallothioneins A and B that are involved in Cu, Zn, Cd and **Hg binding**

- Metallothioneins only bind inorganic metals so Hg²⁺
 - Continuous demethylation of mercury occurs and Hg²⁺ is bound at least temporarily to metallothioneins

Se speciation in trout by LC MS techniques

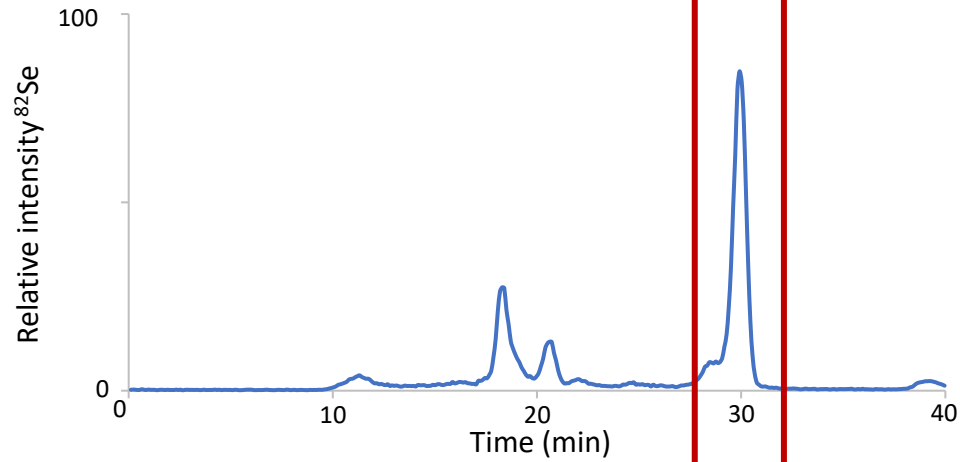
Se (blood)

Size exclusion chromatography-ICPMS

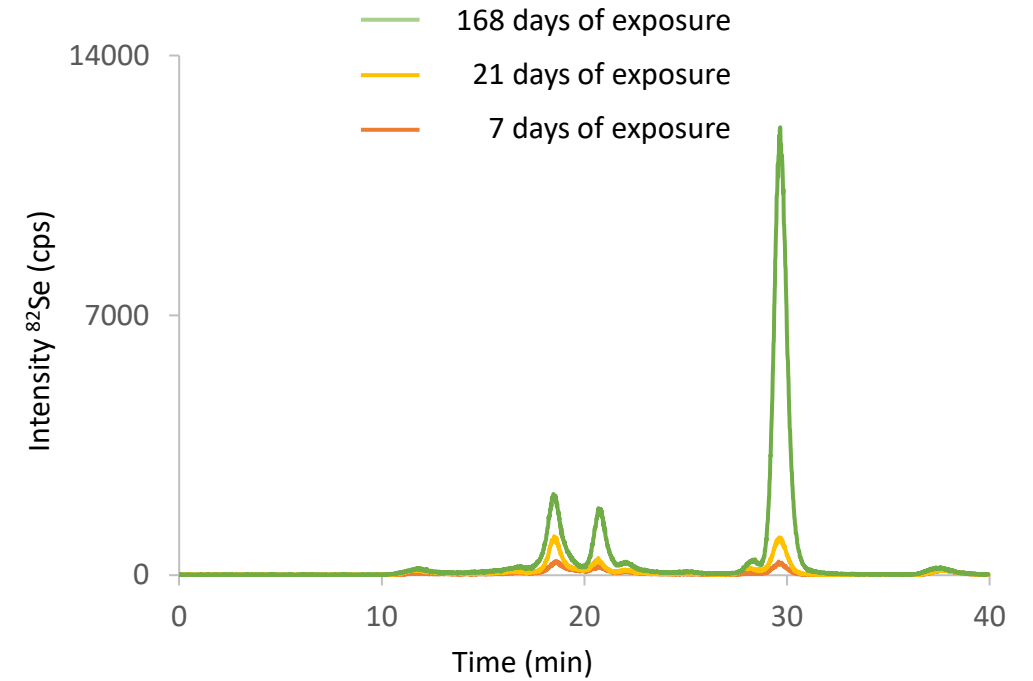
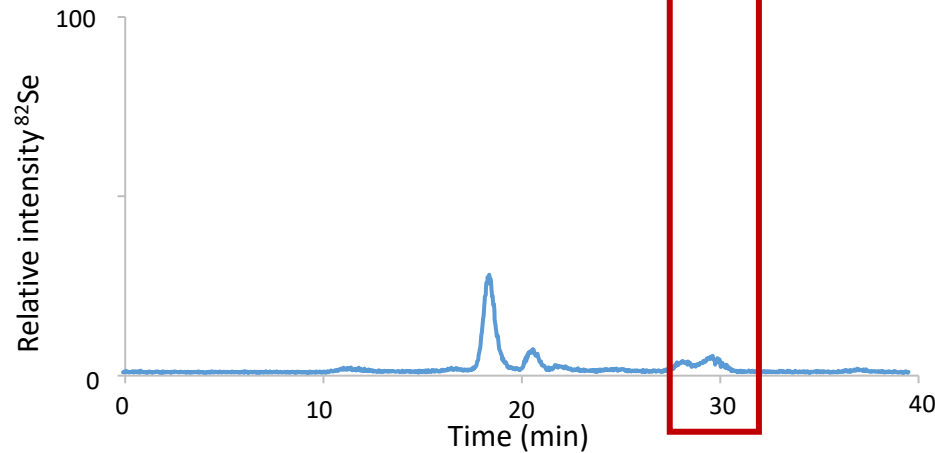


Superdex 200: 600 to 3 kDa

Fed with tuna based diets (84 days)

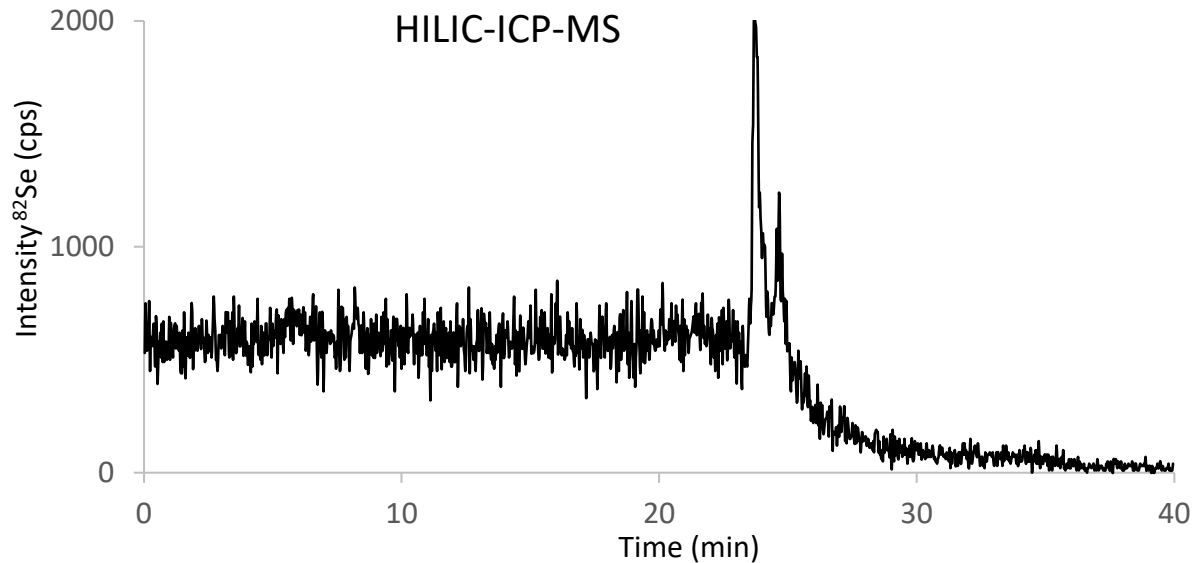


Fed with plant based diets (84 days)

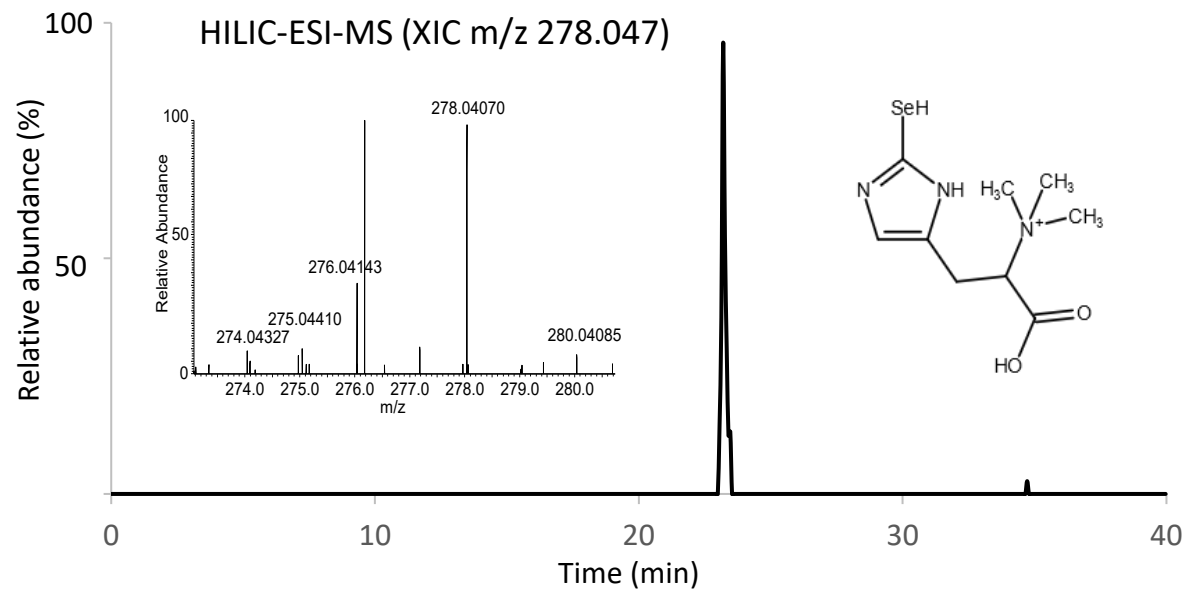


✓ A low molecular weight Se compounds accumulates with time in rainbow trout fed with tuna-based diet.

Se speciation in trout by LC MS techniques



- ✓ Identification of the **antioxidant selenoneine** as the **main Se compounds** in trout fed with tuna based diet after 168 days
- ✓ Increase of selenoneine concentration with the exposure time: **bioaccumulation**



- Selenoneine is only **produced by a few bacteria** having specific biosynthetic genes (SenA/SenB/SenC, Kayrouz 2022, *Nature*).
- **Biomagnification** from bacteria to mammals and **rare occurrence** of bacterial biosynthetic genes are **common characteristic** of MeHg and selenoneine.

Conclusions

- **LC MS** techniques as **ideal tools** to follow Hg (and Se) fate *in vivo*
- Association of **MeHg** to **proteins** and small **thiol**-containing metabolites
- Observed **continuous MeHg demethylation** during growth (increased proportion of Hg²⁺ bound to metallothioneins)
- **Biocummulation of selenoneine** in trout is coherent with the observed high concentrations of this molecule in animal at the top of the trophic chain

Perspectives

- **Proportion between Hg²⁺, MeHg and HgSe** to be investigated in these trout samples
- Could **co-uptake and co-degradation of MeHg and selenoneine result in tienamite (HgSe)** formation to detoxify Hg?

To be continued...

Acknowledgements

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- **To all the collaborators**

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Thank you for your attention!